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Process for the preparation of a edible emulsion

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PROCESS FOR THE PREPARATION OF AN EDIBLE EMULSION

Field of the Invention

The invention relates to a process for the preparation of an edible emulsion having a reduced metal content, an edible emulsion obtainable by such a process and a food product comprising such an edible emulsion. The invention also relates to a skimmed milk powder, a butter milk powder and a whey protein isolate which all have a reduced iron and/or copper content.

Background to the invention

In the case of edible emulsions which comprise an oil phase and an aqueous phase, metal ion catalysed lipid oxidation is known to be one of the major causes of reduced product shelf life. Essentially, the presence of metal ions catalyses the oxidation of both saturated and unsaturated fats promoting the formation of an off-flavour and rancidity. However, polyunsaturated fatty acids are particularly sensitive to metal ion catalysed oxidation. This problem is particularly prevalent in dairy products, that is, products which contain milk or a component or derivative thereof, and, especially, dairy alternatives, that is, spreads, creams and drinks in which the dairy fat and/or protein have been partially or totally replaced by vegetable fat and/or protein.

Milk is a complex biological product that contains many compounds acting as anti- and/or pro-oxidants. Among the pro-oxidants in milk, the transition metal ions of copper and iron are known to play a key role in milk fat oxidation. Although milk contains much more iron than copper (100-900 $\mu g\ l^{-1}$ versus 20-400 $\mu g\ l^{-1}$) and the standard reduction potential of Fe^{3+} suggests that it is a much stronger oxidising agent than Cu^{2+}, different studies reveal that copper is the principal catalytic metal in lipid oxidation. This has been explained by different interactions of the two metals with other milk constituents (e.g. ascorbic acid, thiols or phosphate residues). However, the total endogenous copper content in milk does not appear to be the key factor in oxidation, as it has been found that oxidation via copper already occurs above a threshold value of 0.06 ppb.

Endogenous milk copper and iron form complexes with proteins, peptides, carbohydrates, fats and small molecules like citrate and amino acids via specific and non-specific binding sites. In skimmed milk, 50-65% of the iron is bound to casein, 18-33% to whey proteins and the remainder to non-protein material such as citric acid, orotic acid and inorganic phosphate. Lactoferrin, a whey protein, has two Fe^{3+} binding sites with an affinity of 1×10^{-28} (affinity for Fe^{2+} and copper is much lower), provided sufficient amounts of the cofactor carbonate are present. Copper is also mainly bound to casein, as 1 mole micellar casein can bind 67 mole of copper, whereas 1 mole β-lactoglobulin can only bind 2.5 mole. Serum albumin, a whey protein, is able to bind copper with an affinity of 2×10^{-16} via a specific binding site.

When bound to a protein, binding can be rather specific and the metal ion is not dialysable at neutral conditions. Caseins are known to bind metal ions by their serine bound phosphate (Pser), as well as their tyrosine, glutamic acid and aspartic acid residues. Binding of iron to the Pser group, especially the four high affinity Pser groups of α and β-casein, probably takes place by strong co-ordination bonds as it has been found that neither heat nor pH, nor the presence of Na_2HPO_4, is able to liberate the metal. In contrast, the binding of, for instance, calcium to Pser takes place by much weaker ionogenic bonds. Also, the binding affinity of the metal-amino acid complexes are much lower as it is known that a decreased pH, and subsequently an increased proton concentration, will compete with the metals for the ionisable groups on these cation protein binding sites.

Ligands associated with transition metals can exert a profound influence on the catalytic properties of the bound metal. The formation of iron-casein complexes induces the oxidation of iron from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) state which is known to be less catalytic. Constituents of foods that reduce Fe^{3+} (or Cu^{2+}), like ascorbic acid or thiols, may accelerate the lipid oxidation again. The casein-copper complex is known to inhibit copper catalysed fat oxidation and, recently, it has been found that caseinophosphopeptides can act as natural chelators to inhibit lipid oxidation. On the other hand, the major whey proteins, β-lactoglobulin and α-lactalbumin, bind copper and iron (probably by the carboxylic groups of glutamic acid and aspartic acid) to a much lower extent, whereas their dissociation is influenced by proton concentration.

During the production of dairy spread alternatives, high temperature (up to 85°C) in combination with acidification to low pH will change the protein structure with the result that the catalytic activity of metals on oxidation may be enhanced. Moreover, the presence of milk proteins at the oil-water interface can promote the exposure of metal to the fat phase and thus enhance the oxidation process. In addition, during acidification, (some of) the metals associated with carboxylic groups of glutamic acid and aspartic acid may be liberated and available for further oxidation.

At present, the most efficient solution to the problem of metal ion catalysed lipid oxidation is to include ethylenedinitrilo tetraacetic acid (EDTA) in vulnerable edible emulsions. EDTA is a simple and cost effective metal chelator that eliminates the catalytic lipid oxidation effect caused by both copper and iron. However, legislative restrictions in the field of nutrition and the desire for green labelling of products will reduce the admissibility of this sequestrant in many countries in the coming years. Moreover, other food-grade sequestrants and anti-oxidants are not as effective as EDTA in dairy products and dairy alternatives.

In view of the above, it is an object of the present invention to provide an alternative method for reducing metal ion catalysed lipid oxidation in edible emulsions and thereby increase product shelf life.

Summary of the invention

It has now been surprisingly found that oxidative metals can be removed from the protein-containing starting materials used to form such edible emulsions, especially protein powders, without affecting the functional properties of the proteins. Since such protein-containing starting materials are a major source of oxidative materials, edible emulsions made from such starting materials having a reduced metal content will be less susceptible to metal ion catalysed lipid oxidation.

According to the present invention there is therefore provided a process for the preparation of an edible emulsion having a reduced metal content which comprises an oil phase and an aqueous phase, the process comprising the steps of

(a) providing a starting material containing a protein material;

(b) removing metal from the starting material; and

(c) using the product of step (b) to form an edible emulsion.

In another aspect, the invention provides an edible emulsion obtainable by this process.

In a further aspect, the invention provides a food product comprising such an edible emulsion.

The invention also provides a skimmed milk powder obtainable by steps (a) and (b) of the process of the invention which has an iron content in the range of 1 to 2.5 ppm, preferably 1.2 to 2.4 ppm, based on protein content and a skimmed milk powder obtainable by steps (a) and (b) of the process of the invention which has a copper content in the range of 0.05 to 0.3 ppm, preferably 0.075 to 0.27 ppm, based on protein content.

The invention further provides a butter milk powder obtainable by steps (a) and (b) of the process of the invention which has an iron content in the range of 1 to 15 ppm, preferably 1 to 9 ppm, based on protein content and a butter milk powder obtainable by steps (a) and (b) of the process of the invention which has a copper content in the range of 0.05 to 0.5 ppm, preferably 0.05 to 0.4 ppm, based on protein content.

The invention still further provides a whey protein concentrate obtainable by steps (a) and (b) of the process of the invention which has an iron content in the range of 4 to 6 ppm, preferably 4.4 to 5.2 ppm, based on protein content and a whey protein concentrate obtainable by steps (a) and (b) of the process of the invention which has a copper content in the range of 0.1 to 0.2 ppm, preferably 0.135 to 0.195 ppm, based on protein content.

Detailed description of the invention

In the context of the invention, the terms "fat" and "oil" are used interchangeably. The term oil encompasses both triglyceride oils and diglyceride oils.

For the purpose of the present invention, wt% is defined as weight percent on total product weight unless otherwise indicated.

The invention concerns the preparation of an edible emulsion having a reduced metal content, particularly a reduced content of oxidative metals, especially copper and iron. As mentioned above, the process involves the steps of providing a starting material comprising a protein material, removing metal from the starting material and using the resultant product to form an edible emulsion by conventional methods. Metal removal may be partial or total metal removal. The starting material may also include at least one thickener.

The protein material may be a protein or a fraction or a hydrolysate thereof. The term "protein fraction" refers to a part of a protein which has been obtained by a physical treatment of a protein, for instance, via a physical separation technique. The term "protein hydrolysate" refers to a part of a protein, such as a peptide, which has been obtained by a chemical treatment, for instance, using an enzyme to cut the protein into smaller fragments.

The protein may be any animal or vegetable protein, including fungal or bacterial protein, or a combination thereof. However, it is preferred that the protein is selected from the group consisting of milk proteins, soya protein, pea protein, lupin protein, rice protein, fungal protein and combinations thereof, especially milk proteins, soya protein, pea protein and combinations thereof.

It is particularly preferred that the protein is a milk protein. Suitable sources of milk protein as starting material include whole milk, whole milk powder, skimmed milk, skimmed milk powder, butter milk, butter milk powder, butter serum, butter serum powder, whey, whey powder, whey protein concentrate, whey protein isolate and sodium caseinate. Skimmed milk powder, butter milk powder and whey protein concentrates are especially preferred as starting materials.

Using the process of the invention, it is possible to remove from 25 wt% to 100 wt% of the metal, preferably transition metals, more preferably oxidative metals such as copper and iron, in the starting material. In the case of copper, it is preferred that up to 90% wt%, preferably up to 85 wt%, of the copper in the starting material is

removed. In the case of iron, it is preferred that up to 65 wt%, preferably up to 60 wt%, of the iron in the starting material is removed.

It will be appreciated that the quantity of metal remaining in the starting material after treatment according to steps (a) and (b) of the process of the invention will depend to some exent on the quantity of metal present in the original, untreated starting material. The quantity of metal present in the untreated starting material varies considerably according to the type of starting material. For instance, skimmed milk powder typically contains from 3 to 6 ppm iron and from 0.5 to 1.8 ppm copper based on protein content. However, butter milk powder typically contains from 18 to 25 ppm iron and from 1.5 to 2.5 ppm copper and whey protein concentrate typically contains from 11 to 13 ppm iron and from 0.9 to 1.3 ppm copper based on protein content. If these starting materials are treated according to steps (a) and (b) of the process of the invention, the quantities of iron and copper present can be significantly reduced. Thus, skimmed milk powder can be obtained which has an iron content in the range of 1 to 2.5 ppm, preferably 1.2 to 2.4 ppm, and/or a copper content in the range of 0.05 to 0.3 ppm, preferably 0.075 to 0.27 ppm, based on protein content. Butter milk powder can be obtained which has an iron content in the range of 1 to 15 ppm, preferably 1 to 9 ppm, and/or a copper content in the range of 0.05 to 0.5 ppm, preferably 0.05 to 0.4ppm, based on protein content. Similarly, whey protein concentrate can be obtained which has an iron content in the range of 4 to 6 ppm, preferably 4.4 to 5.2 ppm, and/or a copper content in the range of 0.1 to 0.2 ppm, preferably 0.135 to 0.195 ppm, based on protein content. Such starting materials having a reduced iron and/or copper content also form part of the invention.

Removal of metal, especially oxidative metals such as copper and iron, can be accomplished by any one of a variety of separation techniques known to those skilled in the art or by a combination of such techniques. These techniques can be roughly divided into specific and non-specific ways to remove metal ions. Preferred techniques included filtration, preferably ultrafiltration, dialysis, preferably electrodialysis, and chromatographic separation.

Ultrafiltration techniques are commonly used in the dairy industry to prepare whey protein isolates and lactose and are popular because they are cost effective and can

be easily scaled up. Ultrafiltration is a non-specific way to remove components with a small molecular size, as separation occurs via a membrane with a specific molecular weight cut-off. Consequently, not only the metal ions will be removed when this technique is used, but also other small molecules like lactose and salts, which might have to be re-added afterwards to maintain product quality. As metals are hardly removed under neutral conditions, it may be necessary to carry out ultrafiltration under acidic conditions, at increased temperature, in the presence of a chelator such as EDTA and/or in the presence of a reductant like ascorbic acid. However, use of these additional parameters may affect protein functionality.

Other demineralisation processes developed by the dairy industry to extend their range of products permit a more specific removal of minerals from whey and whey permeates. These processes include nano-filtration ('loose' reverse osmosis), electrodialysis, mineral precipitation and ion-exchange chromatography.

The most specific way to remove metals from milk and whey leaving other small molecular weight substances like lactose and fatty acids undisturbed probably involves chromatographic separation. Various different chromatographic resins with a strong cation binding group linked via a spacer arm to polyacrylamide or agarose (Sepharose) based beads can be used. These have an average size of about 100µm and can be easily separated from milk proteins using a glass filter. Suitable chromatographic resins include hydroxyapetite, sulphopropyl-, thiopropyl- and chelating Sepharose. Immobilised metal affinity chromatography (IMAC) using chelating Sepharose is particularly advantageous as these beads, containing part of an EDTA molecule, are specially designed for metal binding. Also, chromatographic resins containing (immobilised) sulphydryl (thiol) groups are known to specifically bind metals. Sulphopropyl Sepharose and thiopropyl Sepharose are particularly useful in this respect. Thiosuccinylated aminoethyl cellulose can also be used. Bioscavenging of heavy metals from waste water has been accomplished using rice bran and this may also be useful for the separation of metals from milk. Instead of rice bran, numerous other compounds like peanut skins, walnut meal, wool, onion skin, waste tea leaves, etc. can also be used.

The edible emulsion produced by the process of the invention comprises an oil phase and an aqueous phase. The edible emulsion may be an oil-in-water emulsion or a water-in-oil emulsion.

Oil-in-water emulsions comprise an aqueous phase as the continuous phase and an oil phase as the dispersed phase. Also covered are products comprising more than one dispersed (oil) phase and products in which the dispersed oil phase comprises a dispersed phase itself. Such oil-in-water emulsions typically comprise from 1 to 80 wt% fat, preferably 1 to 50 wt% fat, more preferably 5 to 40 wt% fat.

Water-in-oil emulsions comprise an oil phase as the continuous phase and an aqueous phase as the dispersed phase. The fat phase of such a water-in-oil emulsion may constitute up to 95 wt% of the emulsion, preferably no more than 82 wt% of the emulsion. More commonly, the fat phase constitutes up to 60 wt% of the emulsion and, in low fat emulsions which are suitable as low fat spreads, up to 40 wt%.

The emulsion can be used as a final product and may be sold as such. Alternatively, the emulsion may be included in a food product.

The emulsion may be prepared separately and then included in the food product, but alternatively the emulsion is prepared in situ during the preparation of the food product.

Food products in which the emulsion may suitably be incorporated are preferably selected from the group comprising dairy products, such as milk, cheese, yoghurt, cream, ice cream, spreadable products such as margarine, butter, low fat spreads, sauces, dressings and mayonnaise.

Food products including an oil-in-water emulsion preferably include milk, cheese, yoghurt, cream, spreads, mayonnaise, dressings, sauces, ice cream and dairy alternative products. Food products including a water-in-oil emulsion preferably include margarine and low fat spreads.

The oil or fat used may be dependent on the type of product. Preferably, the fat is a vegetable fat, an animal fat, such as a dairy fat, or a combination thereof. Pure vegetable fat or combinations of vegetable fat and dairy fat are especially preferred. In particular, the fat may be either a vegetable oil, animal oil or a marine oil or a combination thereof. The fat is preferably selected from the group consisting of sunflower oil, safflower oil, palm oil, palm kernel oil, soybean oil, coconut oil, dairy fat such as butter fat, rapeseed oil, olive oil, peanut oil or oils extracted from plant or flower material such as rose oil, and combinations thereof. Fully or partially hardened fractions of such oils are also encompassed in the invention. Optionally, the fat may be an interesterified fat blend.

The emulsion may further comprise optional ingredients such as salt, flavour components such as herbs and spices, colourants, emulsifiers, preservatives, acidifying agents, sweeteners, (co)-oxidants such as hydrogen peroxide, and the like. Suitable emulsifiers include monoglycerides (saturated or unsaturated), diglycerides and phospholipids such a lecithins. In addition, the emulsion may contain sterols and/or stanols, preferably phytosterols and/or phytostanols and their corresponding esterified derivatives.

The amount of protein in the emulsion is preferably from 0.05 to 15 wt%, more preferably from 2 to 10 wt%, especially from 2 to 6 wt%.

The invention is further illustrated by the following non-limiting examples.

Examples

Materials and Methods

1. Proteins and sequestrants

Skimmed milk powder (SMP) was obtained from Coberco (SMP-medium heat) and whey protein concentrate (WPC) was obtained from Arla Food (Nutrilac QU7560). Ethylenedinitrilo tetraacetic acid or Titriplex III (EDTA) was obtained from Merck (1.08418), citric acid from Fisher (C/6200/53), $Na_4P_2O_7$ from Merck (6591 pro-

analysis), ascorbic acid from Sigma (A-5960 Sigma ultra 99%) and phytic acid from Aldrich (27,432-1).

2. Element analysis using plasma emission or atomic absorption spectroscopy

Both total and free transition metals in SMP and WPC were analysed using plasma emission spectroscopy analysis. For total metal analysis, the protein powders (0.5g) were digested in 10 ml 65% nitric acid and 0.5 ml 30% hydrogen peroxide in closed vessels in a microwave oven at high temperature (ramp time 15 minutes and hold time 10 minutes at 200°C) and high pressure (55 bar). After digestion, the solution was diluted to 1.4N nitric acid using demineralised water and sprayed into the inductively coupled plasma of a plasma emission spectrometer (Perkin Elmer 3300 DV Inductive Coupled Plasma-Optical Emission Spectrometer). The emission of the individual elements was measured at specific wavelengths (238.20 nm for iron and 324.75 nm for copper) and concentrations were quantified from standard solutions. The amount of free metals was calculated as mg/kg powder or mg/kg protein.

Example 1

Small scale ultrafiltration

General method

A small-scale (15 ml) ultrafiltration unit from Amicon (Centriprep YM3) was used containing a membrane with a molecular weight cut off (MWCO) of 3,000 Dalton. The polystyrene filter units were washed with 1 N HCl overnight and subsequently rinsed with demineralised water and dried. A 6.7% w/v SMP or WPC solution (1g in 15 ml) was added to the retentate chamber and the system was centrifuged (maximally 3,000g) in order to separate the permeate. Upon separation (2 hours), centrifugation was stopped 3 times in order to redissolve sedimented protein. Free metal analysis was carried out using the permeates after the ultrafiltration separation described above. These solutions were directly sprayed into the plasma of a plasma emission spectrometer as described above.

Example 1A

Influence of pH on metal removal

Filtration experiments were carried out as described above at pH values between 3 and 7. The protein solutions were pre-incubated for 3 hours at set pH prior to filtration and no sequestrants were added during this experiment.

It was found that, at pH 4.5-4.7, which corresponds to the pH of dairy spreads, about 6% copper and 0.5% iron is liberated, whereas 32% copper and 3.5% iron is in the free form at pH 3.5.

Similarly, filtration of WPC shows an increased amount of free copper at decreased pH. At pH 3, about 50% of the total copper level is liberated, whereas all the iron is bound.

In summary, ultrafiltration at decreased pH can be used to remove weakly bound copper from SMP and WPC, whereas iron remains strongly bound. Hardly any iron and only half of the copper content is removed upon filtration at pH 3.

Example 1B

Influence of EDTA at pH 7 on metal removal

Both SMP and WPC were filtered at pH 7 in the presence of a concentration series of EDTA, after a 15 minutes pre-incubation period, at room temperature. As the protein content in both powders differs considerably (37% versus 74%), the amount of EDTA is given in w/w on protein. Upon filtration the small metal-EDTA complexes were transported to the permeate. Although results are still expressed in "% free metals", it is actually the metals liberated from protein that are measured. The amount of free copper present in SMP at EDTA < 3% was below the detection limit of the element analysis.

It was found that high EDTA concentrations are required to start liberating copper (at > 3% EDTA) and iron (at > 10% EDTA) from SMP, whereas liberation of both metals from WPC starts at the lowest concentration dose (0.1%). At 13.5% w/w EDTA, 5%

iron and 41% copper is removed from SMP whereas 30% and 65% is removed from WPC.

In order to improve the levels of metal removal, EDTA concentration and pre-incubation time and temperature were increased. It was found that an increased pre-incubation temperature slightly promotes the removal of both iron and copper. At 30% w/w EDTA, 25% iron and 54% copper is removed at room temperature, whereas 39% and 67% are removed at 50°C. Also an extended pre-incubation time (overnight at 4°C) seems to have a small effect, as 41% iron and 61% copper is removed now. Increasing the EDTA concentration up to 90% w/w shows a continuous linear increase of the copper removal, whereas the removal of iron reaches a maximum level of about 40%. Similar results were obtained with WPC.

In summary, about 85% of copper and 40% of iron can be removed from both SMP and WPC in the presence of excess EDTA. An increased removal of copper may be obtained by optimisation of experimental conditions.

Example 1C

Influence of EDTA between pH 3-9 on metal removal

WPC was filtered in the presence of 13.5% w/w EDTA at pH 3, 5, 7 and 9 at room temperature. Solutions were pre-incubated for 15 minutes prior to filtration. The same was done for SMP using 27% w/w EDTA.

It was found that the influence of pH on filtration in the presence of a large amount of EDTA is only limited. Two exceptions were observed. First, filtration of SMP in the presence of EDTA at pH 9 showed a large decrease of the amount of copper and iron removed. Second, the level of iron removed from WPC in the presence of excess EDTA at pH 3 increases up to 60%.

In conclusion, filtration of SMP in the presence of excess EDTA is pH-independent between pH 3-7. Filtration of WPC in the presence of excess EDTA is pH-independent between pH 5-9, whereas increased levels of iron removal occur at pH < 4.

Example 1D

Influence of the combined effect of EDTA and ascorbic acid on metal removal

WPC was filtered in the presence of 13.5% w/w EDTA and/or 36% w/w ascorbic acid at pH 5 at room temperature. Solutions were pre-incubated for 15 minutes prior to filtration. The same was done for SMP using 27% w/w EDTA and/or 71% w/w ascorbic acid. Both EDTA and ascorbic acid concentrations are given in w/w protein, which represents 18 mM EDTA and 100 mM ascorbic acid for both SMP and WPC experiments.

It was found that ascorbic acid itself does remove some iron, whereas no additional copper is removed. The combined presence of EDTA and ascorbic acid during filtration results in an increased removal of iron, compared with the levels obtained in the presence of only EDTA. The amount of iron removed from SMP increases from 35% to 63%, whereas the amount of iron removed from WPC increases from 32% to 50%. Additionally, some extra copper was released from SMP if both EDTA and ascorbic acid were present (29% in the presence of only EDTA and 46% if both are added). No extra copper was removed from WPC.

In conclusion, a beneficial effect with respect to iron removal was obtained upon the simultaneous addition of EDTA and ascorbic acid at pH 5. About 63% of total iron was liberated from SMP and about 50% of total iron from WPC.

Example 1E

Influence of other sequestrants on metal removal

Both SMP and WPC were filtered in the presence of 100 mM sodium pyrophosphate, citric acid or phytic acid at pH 7 at room temperature. Solutions were pre-incubated for 15 minutes prior to filtration.

It was found that the metal affinity of EDTA > sodium pyrophosphate > citric acid > phytic acid. Nevertheless, these other sequestrants may still be useful for removal of metal from protein powders.

Example 2

Chromatographic removal of metals

General Method

Four different chromatographic resins were tested batch-wise, for their ability to specifically bind metal ions from SMP and WPC. These resins include hydroxyapetite, sulphopropyl-, thiopropyl- and chelating Sepharose.

Example 2A

Hydroxyapetite (HA)

Combined anion and cation exchange chromatography was performed using CHT ceramic HA Type II material from Biorad. This form of HA is a robust, chemically pure resin that can be re-used many times. About 10g HA was washed 2 times with demineralised water and 3 times with 10 mM sodium phosphate pH 7 using a sintered glass filter. About 10g of SMP or WPC in 200 ml of the phosphate buffer was incubated for 2 hours at room temperature with the washed HA. The solutions were gently shaken to avoid damage of the chromatographic resin. Finally, the proteins were separated from the chromatographic resin using the sintered glass filter, quickly frozen using CO_2-ice/acetone and freeze-dried.

It was found that HA can be used to remove both iron and zinc from SMP and WPC. The removal of these metals from SMP is comparable with the maximum level obtained using ultrafiltration in the presence of EDTA at pH 7, whereas the removal from WPC is slightly less compared with this ultrafiltration experiment.

Example 2B

Sulphopropyl Sepharose (SP)

Cation exchange chromatography was carried out using SP-Sepharose Fast Flow from Amersham Biosciences. About 10g SP was washed 2 times with demineralised water and 3 times with 10 mM sodium phosphate pH 7 using a glass filter. Exactly

the same batch-wise procedure as described above for HA was applied for the SP-Sepharose.

It was found that as much as 70% of the total iron content of SMP was removed upon incubation with the SO_3 groups of SP Sepharose. This amount is about the same as the maximum amount of iron removal obtained using ultrafiltration in the presence of both EDTA and ascorbic acid at pH 5. The iron content of WPC is only reduced by 7%.

Example 2c

Thiopropyl Sepharose (TP)

Covalent chromatography using an activated thiolated matrix was achieved using TP-Sepharose 6B from Amersham Biosciences. The Sepharose material was first activated into its sulphydryl form by incubation of 1g TP in 4 ml of 0.5 M β-mercaptoethanol and 1 mM EDTA in 0.3 M sodium bicarbonate pH 8.4 for 40 minutes at room temperature. This material was washed with 400 ml 0.1 M acetic acid containing 0.5M NaCl and 100 ml demineralised water using a sintered glass filter. About 10g of SMP or WPC in 150 ml demineralised water was incubated for 2 hours at room temperature with the activated and washed TP. The solutions were gently shaken to avoid damage of the chromatographic resin. The same batch-wise separation procedure was also carried out without the activation step with β-mercaptoethanol. Finally, the proteins were separated from the chromatographic resin using the sintered glass filter, quickly frozen using CO_2-ice/acetone and freeze-dried.

It was found that both TP Sepharose with and without the protecting 2-thiopyridyl group removes about 60-65% iron from SMP. These values are almost equal to the removal accomplished with SP Sepharose. Furthermore, 38% of total copper is removed from SMP if the free thiol material is used. No copper and iron is removed from WPC if TP Sepharose is used, whereas a moderate removal of these metals from WPC is accomplished upon incubation with the free sulphydryl form of TP Sepharose.

Example 2D

Chelating Sepharose (CH)

Immobilised metal chelate affinity chromatography (IMAC) was carried out using CH-Sepharose Fast Flow from Amersham Biosciences. About 5g CH was washed 3 times with demineralised water and 3 times with 50 mM sodium phosphate pH 7 using a sintered glass filter. About 10g of SMP or WPC in 100 ml of the phosphate buffer was incubated for 3 hours at room temperature with the washed CH. The solutions were gently shaken to avoid damage of the chromatographic resin. Finally, the proteins were separated from the chromatographic resin using the sintered glass filter, quickly frozen using CO_2-ice/acetone and freeze-dried.

It was found that iron removal is again more easily accomplished from SMP proteins (25% removal) than from WPC (0% removal) whereas, for removal of copper, it is the other way round (11% versus 27%).

CLAIMS

1. A process for the preparation of an edible emulsion having a reduced metal content which comprises an oil phase and an aqueous phase, the process comprising the steps of

(a) providing a starting material containing a protein or a protein material;

(b) removing metal from the starting material; and

(c) using the product of step (b) to form an edible emulsion.

2. A process according to claim 1 in which from 25 wt% to 100 wt% of the metal in the starting material is removed.

3. A process according to claim 2 in which the metal is an oxidative metal selected from the group consisting of copper and iron.

4. A process according to claim 3 in which the metal is copper and up to 90 wt% of the copper in the starting material is removed.

5. A process according to claim 3 in which the metal is iron and up to 65 wt% of the iron in the starting material is removed.

6. A process according to any one of the preceding claims in which the protein is selected from the group consisting of milk proteins, soya protein, pea protein and combinations thereof.

7. A process according to claim 6 in which the protein is a milk protein and the starting material is selected from the group consisting of whole milk, whole milk powder, skimmed milk, skimmed milk powder, butter milk, butter milk powder, butter serum, butter serum powder, whey, whey powder, whey protein concentrate, whey protein isolate and sodium caseinate.

8. A process according to claim 7 in which the starting material is selected from the group consisting of skimmed milk powder, butter milk powder and whey protein concentrate.

9. A process according to any one of claims 1 to 8 in which the metal is removed from the starting material by filtration, preferably ultrafiltration.

10. A process according to any one of claims 1 to 8 in which the metal is removed from the starting material by dialysis, preferably electrodialysis.

11. A process according to any one of claims 1 to 8 in which the metal is removed from the starting material by chromatographic separation.

12. An edible emulsion obtainable by a process according to any one of claims 1 to 11.

13. A food product comprising an edible emulsion according to claim 12.

14. A food product according to claim 13 in which the edible emulsion is an oil-in-water emulsion.

15. A food product according to claim 14 selected from the group consisting of milk, cheese, yoghurt, cream, spreads, mayonnaise, dressings, sauces, ice cream and dairy alternative products.

16. A food product according to claim 13 in which the edible emulsion is a water-in-oil emulsion.

17. A food product according to claim 16 selected from the group consisting of margarine and low fat spreads.

18. A skimmed milk powder obtainable by steps (a) and (b) of a process according to claim 1 which has an iron content in the range of 1 to 2.5 ppm, preferably 1.2 to 2.4 ppm, based on protein content.

19. A skimmed milk powder obtainable by steps (a) and (b) of a process according to claim 1 which has a copper content in the range of 0.05 to 0.3 ppm, preferably 0.075 to 0.27 ppm, based on protein content.

20. A butter milk powder obtainable by steps (a) and (b) of a process according to claim 1 which has an iron content in the range of 1 to 15 ppm, preferably 1 to 9 ppm, based on protein content.

21. A butter milk powder obtainable by steps (a) and (b) of a process according to claim 1 which has a copper content in the range of 0.05 to 0.5 ppm, preferably 0.05 to 0.4 ppm, based on protein content.

22. A whey protein concentrate obtainable by steps (a) and (b) of a process according to claim 1 which has an iron content in the range of 4 to 6 ppm, preferably 4.4 to 5.2 ppm, based on protein content.

23. A whey protein concentrate obtainable by steps (a) and (b) of a process according to claim 1 which has a copper content in the range of 0.1 to 0.2 ppm, preferably 0.135 to 0.195 ppm, based on protein content.

ABSTRACT

PROCESS FOR THE PREPARATION OF AN EDIBLE EMULSION

The invention provides a process for the preparation of an edible emulsion having a reduced metal content which comprises an oil phase and an aqueous phase, the process comprising the steps of (a) providing a starting material containing a protein material; (b) removing metal from the starting material; and (c) using the product of step (b) to form an edible emulsion. An edible emulsion obtainable by such a process and a food product comprising such an edible emulsion are also provided. In addition, the invention provides a skimmed milk powder, a butter milk powder and a whey protein isolate which are all obtainable by steps (a) and (b) of the above process and all have a reduced iron and/or copper content.

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